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A PRELIMINARY STUDY OF THE NITROGEN NUTRITIONAL STATUS OF MEMBERS OF THE SOUTH AFRICAN PROTEACEAE

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Abstract

An investigation of the free amino compounds of *Leucadendron xanthoconus* and *Protea lepidocarpodendron* leaves indicate very low concentrations of these compounds present. An analysis of the xylem sap of *L. xanthoconus, P. lepidocarpodendron, P. laurifolia* and *Brabeium stellatifolium* also reveals very low levels of nitrogen transporting compounds present, the spectrum of these compounds being dominated by nitrate and ammonium ions, and glutamine. Feeding experiments with KNO_3 and NH_4Cl indicate poor utilisation of additional nitrogen supplies by *L. xanthoconus* shoots, particularly in the case of nitrate feeding. ^{15}N feeding experiments do, however, show limited nitrate reduction capability in the leaves of *L. xanthoconus.*

Uittreksel

'N VOORLOPIGE STUDIE VAN DIE STIKSTOF-VOEDINGSSTATUS VAN LEDE VAN DIE SUID-AFRIKAANSE PROTEACEAE

'n Ondersoek van die vrye aminoverbindinge van *Leucadendron xanthoconus* en *Protea lepidocarpodendron* blare vertoon die aanwesigheid van baie lae konsentrasies van hierdie verbindinge. Oplossings van die xileemsap van *L. xanthoconus, P. lepidocarpodendron, P. laurifolia* en *Brabeium stellatifolium* besit ook 'n baie lae konsentrasie van stikstofvervoerverbindinge, met nitraat- en ammoniakione, en glutamien as die hoofkomponente. Voedingseksperimente met KNO_3 en NH_4Cl vertoon swak benutting van sulke bykomende stikstofbronne deur uitspruitsels van *L. xanthoconus,* veral in die geval van nitraatvoeding. ^{15}N voedingseksperimente het egter bewys dat die blare van *L. xanthoconus* oor 'n beperkte vermoë beskik om nitraat te reduseer.

Although the fynbos of the South Western Cape has been known to civilised man longer than any other vegetation type in Southern Africa, it remains one of the most poorly studied of these types, especially in relation to its ecophysiological relationships. This apparent neglect of the country's most spectacular flora is probably occasioned by three main factors: the complexity of the flora itself; the fact that the topography and soils outside the regions exploited early in the country's history are not favourable for agronomy; and the physical and chemical characteristics of the natural vegetation which make it unsuitable for feeding livestock or for other forms of commercial usage other than cut flowers.

The investigation to be described is a preliminary study of one aspect of fynbos nutrition: the nitrogen status of the family Proteaceae and its comparison with that of non-fynbos families, in an attempt to understand this feature of the

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nutritional strategy of the fynbos employed in coping with the severe nutritional limitations of the soils of their vegetational area.

In this study the following four aspects of the nitrogen nutrition of representative species of Proteaceae have been investigated:—

1. The spectrum of free nitrogen compounds found in the leaves of the species, the quantities of these compounds present and a comparison of these data with that of non-fynbos plants.
2. The spectrum and relative quantities of the translocatory nitrogen compounds responsible for conveying nitrogen from soil to leaf via the xylem stream of the root and stem, and a comparison of these data with relative data obtained for non-fynbos representative species.
3. The effect of feeding additional supplies of nitrate or ammonium ions to the shoots of these plants to ascertain whether these supplies can be utilised and, if so, their influence on nitrogen metabolism in the leaf.
4. The nitrate reducing properties of the leaves of these plants and an assessment of the role of nitrate in leaf nitrogen nutrition.

PLANT MATERIAL and METHODS

Four species of Proteaceae, *Protea lepidocarpodendron* L., *Protea laurifolia* Thunb., *Leucadendron xanthoconus* O. Ktze. and *Brabeium stellatifolium* L. were investigated. *Protea lepidocarpodendron* and *Leucadendron xanthoconus* were growing at 300 m on the N.W.-facing slopes of Orange Kloof, Table Mountain, in soil derived from Table Mountain Sandstone. *Protea laurifolia* and *Brabeium stellatifolium* were growing respectively on the N.E.-facing slopes and the river valley floor of Happy Valley, Bains Kloof in Table Mountain Sandstone derived soil at an altitude of 630 m. Sampling was carried out during April and May 1976, i.e. in late autumn.

Leaf Sampling and Amino Compound Extraction. When leaf samples were removed from plants in the field, they were immediately killed and frozen by immersing them in liquid nitrogen. These frozen samples were returned to the laboratory where known weights of leaf material were homogenized in cold 80% ethanol (100 mℓ 80% ethanol for 5 g leaf material) using an Ultra Turrax homogenizer. The preparations were allowed to extract for 24 h at 0 ° in a sealed flask with occasional shaking, filtered and reduced in volume to 10 mℓ under an airstream.

Xylem Sap Extraction. Xylem sap was extracted from the plants by cutting off leafy twigs in the field and applying pressure to them inside a Scholander Bomb. The xylem sap extruded from the cut surface of the twig stem was collected and frozen immediately.

Soil Nitrate Analysis. Soil samples were collected in plastic bags from under the experimental plants and frozen immediately by immersing them in liquid nitrogen, thus killing the soil bacteria. These were transported to the laboratory, dried at 30 °C and extracted by shaking in distilled water (1:1 weight/volume) for 30 min. Nitrate analysis was performed on soil extracts and xylem sap using an Orion Nitrate Probe coupled to an Orion Model 701 pH Meter.

Amino Compound Analysis. Amino acid and amide content estimates of leaf extracts and xylem sap were made on a Beckman 120C Amino Acid Analyser using the lithium buffer methodology described by Lewis (1975).

Nitrate and Ammonium Ion Feeding. Nitrate ion and ammonium ion solutions were fed to detached shoots of *Leucadendron xanthoconus* in the field as follows:

Shoots were detached from plants at 10h00, their severed ends recut under water to prevent xylem airblocks and placed into beakers containing either a 200 μg N $m\ell^{-1}$ KNO_3 solution or a 200 μg N $m\ell^{-1}$ NH_4Cl solution. These shoots were allowed to photosynthesize in sunlight alongside the plants from which they had been detached for 8 hours, when they were frozen and extracted as described about. Reference shoots were removed from the plants for analysis at the beginning of the experiment and at the end of the 8-hour period.

A further experiment in which nitrate was fed continuously to detached shoots of *L. xanthoconus* for 24 h was performed in a "Conviron" controlled environment cabinet under constant light (16 000 lux), a temperature of 16 °C and a relative humidity of 80 %.

The duplicated results of these feeding experiments are reported in Table 3.

^{15}N Isotope Studies on Nitrate Utilisation. In this experiment a leafy shoot of *L. xanthoconus* was fed a solution of 200 μg ^{15}N $m\ell^{-1}$ K $^{15}NO_3$ for 24 h in a controlled environment cabinet under the conditions described above, to determine whether the shoots of this plant are able to reduce and utilise nitrate.

After extraction, the amino compounds of the extract were separated on a 150 × 1,8 cm Beckman M-84 ion-exchange resin column and collected in a fraction collector. The amino compound samples were prepared for ^{15}N atomic emission analysis by Kjeldahl digestion and ammonia distillation. These analyses were performed on a Statron (Packard) atomic emission spectrophotometer following oxidation of the ammonia with sodium hypobromite by the method of Faust (1967).

RESULTS AND DISCUSSION

Free Amino Compounds in the leaves of P. lepidocarpodendron *and* L. xanthoconus

The levels of free amino compounds occurring in these leaves are indicated in Table 1. Also shown in this table are the relative figures for a non-fynbos plant typified by *Datura stramonium* L. growing in fertilised agricultural soil near Cape Town. The same amino-compound extraction procedure was used for *Datura* as for the fynbos plants.

From this table it can be observed that the free amino acid and amide levels in the leaves of *P. lepidocarpodendron* and *L. xanthoconus* are extremely low when

TABLE 1

The concentrations (μmol g fr. wt.$^{-1}$) of the free amino compounds of the leaves of two members of the Proteaceae compared with *Datura stramonium*.

Amino Acids	*Leucadendron xanthoconus*	*Protea lepidocarpodendron*	*Datura stramonium*
Aspartate	0,13	0,16	2,67
Threonine	0,10	0,07	1,16
Serine	0,30	0,26	0,94
Asparagine	0,04	0,07	0,69
Glutamate	0,30	0,36	3,68
Glutamine	0,03	0,09	1,45
Proline	—	—	—
Glycine	0,26	0,25	0,08
Alanine	0,10	0,13	0,48
Valine	0,01	0,02	0,01
Cystine	—	—	—
Methionine	—	—	—
Isoleucine	0,03	0,03	0,26
Leucine	0,03	0,03	0,07
Tyrosine	0,02	0,02	0,13
Phenylalanine	0,02	0,03	0,04
Lysine	0,25	0,15	0,13
Histidine	0,10	0,05	0,04
Arginine	0,01	0,01	1,00

compared with those of *D. stramonium*, with serine, glycine and glutamate dominating the amino acid spectrum.

This low free amino compound level is an indication that the level of N metabolism activity in these fynbos plants is low in comparison with that of plants adapted to living under more favourable soil N nutritional conditions.

Free Amino Compounds in the Xylem Sap of P. lepidocarpodendron, P. neriifolia, L. xanthoconus *and* B. stellatifolium

Table 2 shows a quantitative comparison of the amino compounds, nitrate and ammonium ions being transported from root to leaf via the xylem sap in the four Proteaceae species with those of *D. stramonium*. In the Proteaceae species the extremely low concentrations of nitrogen compounds found in the xylem sap are immediately obvious, indicating the very restricted supply of nitrogenous nutrients to the shoot in comparison with a plant adapted to a more favourable nutritional environment.

In all four fynbos plants, glutamine is the dominant amino compound present in the xylem sap, reflecting the importance of this amide as a translocator of reduced nitrogen from root to leaf in many plants. The major utilisable nitrogen carrier appears to be ammonium ions.

The unreduced nitrogen supply to the leaf in the form of nitrate present in the xylem sap, is greater than the total reduced nitrogen supply (amino compounds +

TABLE 2

The free amino compounds of the xylem sap of 4 species of Proteaceae compared with *Datura stramonium*.

Amino Compounds	*Leucadendron xanthoconus* µmol N mℓ$^{-1}$ xylem sap	*Protea lepidocarpodendron* µmol N mℓ$^{-1}$ xylem sap	*Brabeium stellatifolium* µmol N mℓ$^{-1}$ xylem sap	*Protea laurifolia* µmol N mℓ$^{-1}$ xylem sap	*Datura stramonium* µmol N mℓ$^{-1}$ xylem sap
Lysine	0,015	0,00	Trace	0,001	8,73
Histidine	Trace	0,001	Trace	0,002	6,42
Ammonia	0,198	0,174	0,030	0,054	23,91
Arginine	Trace	Trace	0,001	Trace	2,98
Aspartate	0,002	0,012	0,002	0,003	0,883
Threonine	Trace	0,004	0,001	Trace	1,310
Serine	0,003	0,010	0,002	0,001	0,180
Asparagine	Trace	0,003	0,002	Trace	3,297
Glutamate	0,002	0,008	0,002	0,002	2,063
Glutamine	0,012	0,040	0,030	0,014	25,31
Glycine	0,003	0,002	Trace	Trace	0,05
Alanine	0,001	0,005	Trace	0,001	0,190
Valine	—	—	—	—	4,80
Cystine	—	—	—	—	—
Methionine	—	—	—	—	—
Isoleucine	Trace	Trace	Trace	Trace	1,89
Leucine	Trace	0,001	Trace	—	2,47
Tyrosine	—	—	—	—	0,24
Phenyalanine	—	—	—	—	0,393
Nitrate N	0,800	1,200	0,300	0,400	173,0
Total N	1,036	1,463	0,370	0,478	258,12

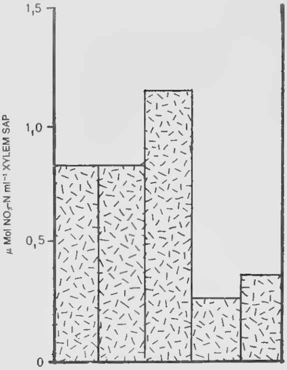

FIG. 1 (a)
Nitrate content of xylem sap of *Leucadendron xanthoconus* (LX) (two samples) *Protea lepidocarpodendron* (PL) *Brabeium stellatifolium* (BS) *Protea laurifolia* (PN)

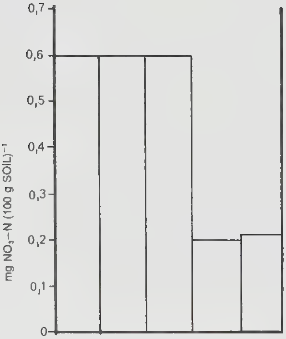

FIG. 1 (b)
Nitrate content of soils in which the plants were growing.

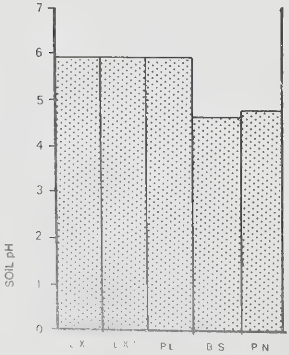

FIG. 1 (c)
pH of soils in which the plants were growing.

ammonium), but there is strong doubt as to whether this supply can be utilised by the leaf as the experiments to be described indicate.

The quantity of nitrate present in the xylem sap appears to be directly related to soil nitrate content and soil pH. Fig. 1 shows the correlation between xylem nitrate content, soil nitrate content and soil pH. The lower nitrate levels in the low pH Bains Kloof soils are probably a manifestation of the well known phenomenon of nitrifying bacteria inhibition in acidic soils.

The Effect of Nitrate and Ammonium Ion Feeding on Nitrogen Metabolism in Photosynthesizing shoots of L. xanthoconus

The results of the nitrate feeding experiments in which 200 μg N $m\ell^{-1}$ was fed to photosynthesizing shoots via the xylem stream in the form of potassium nitrate are reported in Table 3. An inspection of this table shows no significant differences between the aminograms of experimental plants with their controls after a feeding period in the light of 8 h. After a 24 h feeding period there is a slight increase in the level of the N-incorporating amino acid, glutamic acid, indicating a small degree of nitrate ion utilisation by the shoots of *L. xanthoconus*.

The results of the ammonium feeding experiments in which 200 μg N $m\ell^{-1}$ was fed to photosynthesizing shoots in the form of ammonium chloride are also shown in Table 3. An inspection of this table shows a considerable increase in the glutamine levels of the experimental plants compared with the control plants, indicating that the additional ammonium supply can to a large extent be incorporated into shoot metabolism. Glutamine has been shown to be an important contributor of reduced nitrogen to plant amino acid synthesis via the glutamate synthase pathway (Lee & Miflin, 1974) and is known to accumulate in plants receiving high nitrogen nutrition (Lewis & Berry, 1975).

Nitrate Reduction by Shoots of L. xanthoconus

The ^{15}N enrichment of three important free amino acids of the leaf of *L. xanthoconus* after feeding $K^{15}NO_3$ (99 atom % excess) at the 200 μg N $m\ell^{-1}$ level for 24 h is shown in Table 4. These enrichments are extremely low for a 24 h feeding period and confirm the poor nitrate processing properties of *L. xanthoconus* shoots. It nevertheless appears that the shoots do have limited nitrate

TABLE 4

^{15}N enrichment of glutamate, aspartate and threonine in leaves of *L. xanthoconus* fed $K\,^{15}NO_3$ (200 μg ^{15}N $m\ell^{-1}$) through the xylem stream for 24 h.

	^{15}N CONC. (ATOM % EXCESS)
GLUTAMATE	6,3
ASPARTATE	5,0
THREONINE	1,6

TABLE 3

Effect of feeding KNO_3 and NH_4Cl solutions at 200 μg N mℓ^{-1} concentrations on free amino compound levels* (μmol g fr. wt.$^{-1}$) in leaves of *L. xanthoconus.*

	CONTROL (from shrub) (duplicate results)		KNO_3-fed		(duplicate results)		NH_4Cl-fed (duplicate results)	
	8 h	8 h	8 h	8 h	24 h	24 h	8 h	8 h
Aspartate	0,23	0,16	0,16	0,20	0,10	0,15	0,26	0,22
Threonine	0,04	0,05	0,06	0,07	0,05	0,04	0,07	0,12
Serine	0,15	0,07	0,19	0,25	0,04	0,12	0,40	0,30
Asparagine	0,02	0,02	Trace	Trace	Trace	Trace	0,03	0,15
Glutamate	**0,27**	**0,19**	**0,17**	**0,20**	**0,36**	**0,51**	**0,47**	**0,64**
Glutamine	**0,03**	**0,02**	**Trace**	**Trace**	**0,05**	**0,10**	**1,99**	**1,33**
Proline	—	—	—	—	—	—	—	—
Glycine	0,07	0,02	0,06	0,09	Trace	0,05	0,04	0,01
Alanine	0,05	0,01	0,06	0,08	0,20	0,20	0,19	0,04
Valine	—	—	—	—	—	—	—	—
Cystine	—	—	—	—	—	—	—	—
Methionine	—	—	—	—	—	—	—	—
Isoleucine	Trace	—	Trace	0,01	—	—	0,01	Trace
Leucine	—	—	—	0,01	—	—	0,01	Trace
Tyrosine	—	—	—	—	—	—	—	—
Phenyalanine	—	—	—	—	—	—	—	—

*Recognised reduced-N accepting compound in bold type.

reducing properties which may be further induced after prolonged exposure to high nitrate concentrations.

CONCLUSIONS

If the species investigated in this report can be considered representative of the Proteaceae, it would appear that this family has adapted to the poor nutrient status of the soils in which it grows by evolving a low intensity nitrogen metabolism to correspond with a slow growth habit. It would also appear that the shoots of *L. xanthoconus* are unable to utilise to any great degree high levels of nitrogen supplied to them, especially if the nitrogen is provided in the form of nitrate. Other workers (Groves & Keraitis, 1977) have shown, indeed, that high levels of nitrogen (250 μg N mℓ^{-1}) and phosphorus feeding can prove fatal to *Banksia serrata*, an Australian member of the Proteaceae, confirming the restricted nitrogen processing potential of the family.

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